46. The vector of Claim 45, which is pTRANS.

47. A bacterial artificial chromosome (BAC) vector consisting essentially of a high copy origin of replication flanked by cleavage sites for a restriction enzyme, wherein cleavage of the vector with the restriction enzyme leaves single base extensions for cloning and removes the high copy origin of replication.

48. The vector of Claim 47, further consisting essentially of a BST X1 site.

49. The vector of Claim 48, which is pBacTA.PUC2.

REMARKS

The foregoing amendment and the following remarks are submitted in response to the Office Action of May 9, 2001. In this amendment, Applicants have canceled Claims 19-27 in order to comply the restriction requirement made final by the Examiner on May 9, 2001. Applicants have also made merely formal amendments to the instant Specification and Claims 1, 11, 14, and 16, and have added new Claims 28-49. Support for these formal amendments, as well as new Claims 28-49, can be found generally throughout the instant Specification, and in Claims 1-27 as filed. Moreover, Applicants submit herewith a new executed Declaration and Power Attorney which includes the citizenship of all inventors, a sequence listing, a computer readable form (CRF) of the sequence listing, and a statement pursuant to 37 CFR 1.821-1.825. Also, attached hereto is a marked-up version of the changes made to the Claims by the instant Amendment. The attached page is captioned "**Version With Markings To Show Changes**

Made."

The Invention is Enabled

Claims 13, 15, and 18 have been rejected 35 U.S.C. § 112, first paragraph as not being enabled. The Examiner has asserted that Claim 13 is drawn to a pTRANS-SacB vector, Claim 15 is drawn to a pTRANS vector, and Claim 18 is drawn to a pBacTA.PUC2 vector. However, the Examiner believes it is not clear in the instant Specification that these particular vectors are freely available or can be reproducibly isolated from nature. Thus, it is the opinion of the Examiner that a biological deposit of these vectors for patenting purposes is required.

Applicants respectfully traverse this rejection. It is submitted that materials used in the production of these vectors are readily commercially available, and that the instant Specification clearly provides teachings that enable one of ordinary skill in the art to manufacture these vectors using routine laboratory techniques. For example, with respect to the plasmid pTRANS-*sacB*, the instant Specification readily explains at page 1, lines 15-28 that:

> In a preferred embodiment, the transposon plasmid is pTRANS-*sacB* (Fig. 2). A commercially available transposon plasmid, pGPS1, (New England Biolabs, Fig. 1) was used as a starting point in the construction of pTRANS-*sacB*. pGPS1 contains a defective origin of replication which is non-functional in wildtype *E. coli*. It also contains universal primer sites for DNA sequencing at both ends of the transposable element. Several modifications have been made to pGPSI to generate pTRANS-*sacB*. A moderate copy origin of replication (the pBR322 ori) has been introduced into the transposon ("*trans*"). Universal primer sites for DNA sequencing (from pGPS1) are encoded at both ends of *trans* and a T7 promoter, directed "outward" (i.e., away from the transposable element), it encoded on one end. The plasmid also contains the *B. subtilis sacB* gene for counterselection. *In vitro* transposition of *trans* into a single copy BAC vector introduces a moderate copy ori, thereby increasing the copy number of the target BAC vector.

Further instruction regarding the manufacture of this vector is set forth on pages 18-19 of the instant Specification.

It is also explained on page 4, lines 3-5 of the instant Application that pTRANS contains (a) a transposable element containing a pBR322 origin of replication, (b) a kanamycin resistance gene, and (c) a T7 promoter. Moreover, "Plasmid pTRANS is identical to pTRANs-*SacB* except for the absence of the counter-selectable marker, the *B. subtilis sacB* gene." (page 6, lines 5-7 of the instant Specification).

Furthermore, with respect to the vector pBacTA.PUC2, it is explained that this vector is very similar to BACTAPUC1 (pBTP1), except that pBacTA.PUC2 contains a BstXI restriction site. The starting material for both of these vectors is the vector pBeloBAC11, which Applicants explain on page 17, line 4, is a ***commercially available plasmid***. Moreover, detailed instructions regarding the construction of these two vectors is set forth on pages 19-20 of the instant Application.

It is respectfully submitted that MPEP § 2404.02 makes clear, *inter alia*,

> No deposit is required, however, where the required biological materials can be obtained ***from publicly available material*** with only routine experimentation and a reliable screening test. *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977); *Ex Parte Hata*, 6 USPQ2d 1652 (Bd Pat. App. & Int. 1987).

(emphasis added).

Such is the case with the specifically claimed plasmids. The starting materials for these plasmids are publicly available. Moreover, the detailed instructions set forth in the instant Specification readily permit a skilled artisan to manufacture a plasmid of the instant Invention using only routine laboratory techniques. Thus, it is respectfully submitted that plasmids of the

instant Invention need not be deposited, and this rejection is obviated.

The Invention is Definite

Claims 1-18 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. The Examiner has asserted that Claims 1, 9, 11 and 14 recite the word "containing" which, in the Examiner's opinion, is vague and indefinite because it is not legally defined as open or closed language. The Examiner has also asserted that the phrase "improved BAC vector" recited in Claim 16 is indefinite because, in the Examiner's opinion, it is unknown over what the vector is improved.

Applicants respectfully traverse this rejection. Claims 1, 9, 11, and 14 have been amended to recite the term "comprising" rather than "containing." Moreover, Claim 16 has been amended so that it no longer recites the term "improved." Hence, it is respectfully submitted that this rejection is obviated.

The Invention is Novel

Claims 1-12 and 14 have been rejected under 35 U.S.C. §102(b) as being anticipated by the teachings in U.S. Patent 5,645,991 (the '991 patent). The Examiner has asserted that the '991 patent teaches a vector comprising a bacterial transposon, γδ, within which is an origin of replication (ori) from pUC19 plasmid (ColE1), which is a high copy number ori. The Examiner also believes that the vector disclosed in the '991 patent further comprises a T7 promoter, an SP6 promoter, a kanamycin resistance gene, a tetracycline gene, an ampicillin, and a *sacB* and a *strA* gene as counterselectable markers.

Applicants respectfully traverse this rejection, and respectfully submit that significant differences exist between the teachings of the '991 patent and the instant Invention. For

example, the '991 patent teaches a plasmid comprising a transposon that is ***flanked by two*** contraselectable genes that are different from one another. In particular, it is specifically explained in column 3, lines 28-36 that:

> It is [sic] further object of the present invention to provide a plasmid having a specialized mini-γδ transposon containing a plasmid replication origin between the ends of the transposon, and ***bracketed by pairs of conditional lethal and selectable genes in a particular configuration in the plasmid*** in order to efficiently select deletion derivatives in which the deletion extends into the cloned fragment in either one direction or the other, and does not extend beyond the cloned fragment (emphasis added).

In stark contrast, a plasmid of the instant Invention does not require ***two*** contraselectable marker genes. Indeed, on page 12, lines 3-4 of the instant Specification, Applicants specifically state, "The plasmids ***may*** also contain ***a*** counterselectable marker, which is outside the transposable sequence (emphasis added)."

Another significant difference between a plasmid of the instant Invention and a plasmid taught in the '991 patent concerns the origin of replication. In particular, the instant Invention teaches that the ori contained in the plasmid have "...a moderate or high copy number." (page 3, lines 4-5; Claim 1). In contrast, the copy number of the origin of replication in a plasmid of the '991 patent is unimportant. In particular, it is explained in column 6, lines 2-7 of the '991 patent that:

> ***Which origin of replication is used is not critical***: for some purposes it may be advantageous to use an origin that maintains its host plasmid in high copy number or low copy number, or to use an origin that maintains its host plasmid under low copy number in some growth conditions, and under high copy number in other growth conditions (emphasis added).

Yet another significant difference between the teachings of the '991 patent and the instant Invention, particularly as set forth in pending Claims 11 and 12, involves the location of antibiotic resistance and/or contraindicative genes in the vector. More specifically, '991 patent teaches that the transposon be ***flanked*** by these two genes. However, no such requirement is set forth in the instant Invention.

Hence, for the foregoing reasons, it is respectfully submitted that the subject matter of Claims 1-12 and 14 is novel with respect to the teachings of the '991 patent, and this rejection should be withdrawn.

Furthermore, Claims 1-7, 10 and 14 have been rejected under 35 U.S.C. §102(e) as being anticipated by the teachings of U.S. Patent 5,948,622 (the '622 patent). The Examiner has asserted the '622 patent teaches a vector comprising a Tn5 bacterial transposon within which is the ColE1high copy number origin of replication. The Examiner also believes that the vector taught in the '622 patent further comprises an antibiotic gene such as kanamycin or ampicillin resistance, the T7 promoter, and a second selectable marker that can be distinguished from the first selectable marker.

Applicants respectfully traverse this rejection. Figure 1 of the '622 patent shows a vector having a transposable element containing ***three*** selectable markers, e.g., Cam^R and Tet^R, as well as a Kan^r gene located outside of the transposable element. Moreover, in column 11, lines 11-14 of the '622 patent, it is specifically explained "The transposable portion also encodes an origin of replication operable in a selected host cell and a first selectable marker operable in a host cell, ***nearby to the origin of replication*** (emphasis added)."

As explained above though, Claims 1-7, 10 and 14 of the instant Application do ***not*** recite

that a vector of the instant Invention contain ***three*** counterselective markers. Thus, it is respectfully submitted that the subject matter of these Claims is clearly novel with respect to the teachings of the '622 patent, and this rejection is obviated.

In addition, Claims 1, 2, 5-7, 14, 16 and 17 have been rejected under 35 U.S.C. §102(b) as being anticipated by the teachings of Marsch-Moreno *et al.* (1998 Plasmid 39:205-214). The Examiner has asserted that Marsch-Moreno *et al.* teach a vector comprising Tn5-derived mobile element within which is a high copy number ColE1 origin or replication. Moreover, the Examiner believes the vector taught by Marsch-Moreno *et al.* comprises a kanamycin resistance gene, transcription control sequences for the tnp, tet and neo genes, *Bst XI* site, and an origin or replication derived from pBR322 that is flanked by *Nci I* sites.

Applicants respectfully traverse this rejection. In the instant Specification, it is explained that a vector of the instant Invention comprises, *inter alia*, a transposon that has an origin of replication. In stark contrast, a vector taught by Marsch-Moreno *et al.* does not utilize a transposon per se, but rather a "defective" transposon. More specifically, it is explained on pages 212-213 of Marsch-Moreno *et al.* that in their vector, "...the transposition element has been rendered 'defective' by placing the transposase gene ***outside*** the borders [of the transposon] (emphasis added)."

Another significant difference between a vector of the instant Invention and a vector described by Marsch-Moreno *et al.* involves the very nature of the transposase gene. In particular, on page 208, Marsch-Moreno *et al.* explain:

> Also, to have an efficient mutagenesis system and in an attempt to improve on the transposition frequencies previously reported for *P.s. phaseolicola*, we used a ***mutated*** transposase *tnp* MA56

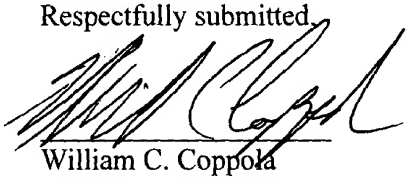
> EK345, which has been reported to present a higher activity in *trans* and to increase transposition about 60-fold in *E. coli* (emphasis added)....

It is well established in patent law that "...for anticipation under 35 U.S.C. § 102, the reference must teach ***each and every aspect*** of the claimed invention either explicitly or impliedly. Any feature not directly taught must be inherently present (emphasis added)." (M.P.E.P. § 706.02(a)). Due to the significant differences between a vector of the instant Invention and vectors described by Marsch-Moreno *et al.*, it is respectfully submitted that the instant Invention is novel with respect to the teachings of Marsch-Moreno *et al.*, and this rejection is obviated.

CONCLUSION

Applicant respectfully requests entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited.

Respectfully submitted,



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Version With Markings To Show Changes Made

What is underlined has been added, and what is bracketed has been removed.

IN THE SPECIFICATION:

Fourth Paragraph of page 11 through the first paragraph of page 12:

Additionally, the plasmids may contain one or more transaction control sequences. One such sequence should be found within the transposable sequence, such that when the transposon hops into the target plasmid, it carries along with it the transcription control sequence. An exemplary sequence is the T7 promoter, but any promoter or enhancer that is functional in prokaryotic cells may be used. Useful promoters include, but are not limited to, *lac* (*E. coli*), *trp* (*E. coli*), *araBAD* (*E. coli*), *tac*, hybrid, (*E. coli*), *trc*, hybrid (*E. coli*), *lpp-lac* hybrid (*E. coli*), PL ([□] λ), *T7-lac* operator and [□] λPL, PT7 ([□] λ, T7).

Page 19, first paragraph:

Example 2: Construction of BAC vectors

1. BACTAPUC1 (pBTP1)- The fist version of the vector, pBTP1, combines pBeloBAC with a high copy PUC-based vector. As shown in Fig 1, insertion of an entire PUC plasmid into the cloning site accomplishes several things. First, it simplifies the purification of the vector prior to cloning by virtue of the high copy ori within the PUC insert which drives the copy number to >100 copies/cell. Second, by using a unique oligonucleotide adapter, we have introduced additional cloning sites. This includes the ability to utilize cloning based on single base extensions. Thermostable polymerases such as Taq have a nontemplate-dependent activity which adds a single deoxyadenosine (A) to the 3' end of DNA. This single extended DNA will

ligate efficiently with a vector that has corresponding deoxythymidine (T) ends. By incorporating a restriction site with internal degenerate internal bases, such as AhdI (GACNNNNNGTC (SEQ ID NO:1)), we can create a vector which, when cut with AhdI, leaves a single T on each end. By treating the genomic DNA with a series of polymerases (T4 and Klenow for blunting followed by Taq to add a single A) DNA can be directly cloned without the need for partial restriction digestion. This latter point is key since cloning by partial restriction digestion will decrease the average insert size of the library by at least half (see below).

Page 20, first paragraph:

to cloning will decrease the average insert size of the final library. In addition, since the average size of the input DNA is in the range of 150 kb before digestion and drops to 75 kb after partial digestion, it is likely that an increasing bias will occur as we attempt to clone [clones] fragments above 80-100kb. This will be dependent on the enzyme used for digestion and the number of sites in the DNA. Therefore, alternate strategies for cloning directly become key in constructing high quality libraries (see Table 1). The single base extension cloning system described above is one way to circumvent this problem. However, although the efficiency of cloning is greater than blunt-end cloning, it is not as high as with multiple base ligation. Also, the addition of the A tail is not 100% efficient, so not all DNA will be ligatable. An alternate approach is to incorporate non-palidromic adapters with 4-base pair overhangs which will greatly increase the efficiency of cloning. Figure 9 (pBTP3) illustrates an example of one such system which uses a second degenerate restriction enzyme, BstXI (CCANNNNNNTGG) (SEQ ID NO:2). In this system, adapters with non-homologous ends (5' CACA 3') are ligated onto blunt-end genomic DNA.

These adapters will not self-ligate but will only anneal with corresponding ends which are generated in the vector by inserting the appropriate BstXI restriction site (5' GTGT 3').

IN THE CLAIMS:

1. (Amended) A vector for increasing the copy number of plasmids, comprising a transposable element comprising [containing] a moderate or high copy number origin of replication capable of *in vitro* transposition into a target plasmid.

9. (Amended) A vector for increasing the copy number of plasmids comprising:

(d) a transposable element comprising [containing] a moderate or high copy number origin of replication;

(e) an antibiotic resistance gene; and

(f) a counterselectable marker.

11. (Amended) A vector for increasing the copy number of plasmids comprising:

(d) a transposable element comprising [containing] a pBR322 origin of replication;

(e) a kanamycin resistance gene; and

(f) a *B. subtilis sacB gene.*

14. (Amended) A vector for increasing the copy number of plasmids comprising:

(a) a transposable element comprising [containing] a moderate or high copy number origin of replication;

(b) an antibiotic resistance gene; and

(c) a transcription control sequence.

16. (Amended) A bacterial artificial chromosome (BAC) [An improved BAC] vector comprising a high copy origin of replication flanked by cleavage sites for a restriction enzyme, wherein cleavage of the vector with the restriction enzyme leaves single base extensions for cloning and removes the high copy origin of replication.